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THE EFFECTS OF CHANGES IN DIET ON THE INCIDENCE, DISTRIBUTION AND NUMBERS OF CERTAIN INTESTINAL PROTOZOA OF FROG AND TOAD TADPOLES *

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So far as known to the writer no studies have been published on the relations between the diet of an animal and the incidence, distribution and numbers of the intestinal protozoa with which it may be infected. In the following pages are recorded the results of some investigations in this field. The host animals used were principally tadpoles of the green frog, *Rana clamitans*, and of the leopard frog, *R. pipiens*, and toad, *Bufo lentiginosus americanus*. The protozoa studied were two ciliates of the genera *Opalina* and *Nyctotherus*, and two flagellates of the genera *Trichomonas* and *Hexamitus*. Other protozoa are more or less regular inhabitants of the digestive tract of these tadpoles but the four mentioned are usually numerous and easily found and are often present in 100 per cent. of the tadpoles examined. Tadpoles of the green frog were selected for special study for several reasons: (1) they do not metamorphose the first season and hence can be obtained, at least in the southern part of their range, at any time of the year; (2) they are hardy and can be subjected to severe experimental conditions without great mortality; (3) they are usually well infected with intestinal protozoa, and (4) one type of protozoon, an *Opalina*, is an inhabitant of the tadpole but not of the adult (Metcalf, in litt.),† a condition that affords an interesting problem for investigation. In certain other species of frogs and toads the infection with *Opalina* appears to be continuous from tadpole to adult.

The observations and experiments described below involve a study of (1) changes that occur in the protozoan fauna of the green frog during the tadpole stage and the process of metamorphosis; (2) factors

* This is the first of a series of papers on the relation between diet and intestinal protozoa from the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University. The writer is indebted to Dr. H. D. Reed for many courtesies during the summer of 1922 while working in the Zoological Laboratory at Cornell University.

† Kudo has recently reported an *Opalina* from this species but this apparently is of rare occurrence.

that bring about the loss of *Opalina* in the adult frog, and (3) the failure of the adult green frog to become reinfected. The incidence of infection, distribution within the alimentary canal, and numbers of intestinal protozoa present were obtained for several stages in the growth and metamorphosis of the normal tadpole; some experiments on the effects of starvation on the protozoan fauna were carried out; and the results of various changes in diet were determined.

I. *Observations on the Course of Infection with Intestinal Protozoa—During the Growth and Metamorphosis of Frog and Toad Tadpoles.*—As stated above, *Opalina*, with one exception, has not been recorded from adults of the green frog, *Rana clamitans*. The course of infection with this genus of protozoon during the growth and metamorphosis of the larvae of this species has not been reported. It is of interest in connection with the experimental work described below to determine at what time the tadpoles become infected and when and why they lose their infection. The writer examined a number of larvae of the green frog that were collected on July 25, 1922, about ten days after hatching. Every one of them was infected with *Opalina*—some of them abundantly infected with this form—as well as with *Nyctotherus*, *Trichomonas* and *Hexamitus*. The tadpoles of *R. clamitans* are not gregarious but scatter widely soon after hatching, hence infection by close association with one another seems improbable. The high rate of infection and large numbers present at the early date of examination, therefore, indicates a strong susceptibility to infection of the tadpole. The larval life of the green frog extends over two seasons, and in the vicinity of Baltimore it is possible to obtain tadpoles during the winter months. Examinations of many specimens collected at various times of the year has shown that *Opalina* as well as the other three genera named above are inhabitants of the rectum of *R. clamitans* throughout larval life.

In 1922 an opportunity was afforded to study the intestinal protozoa of metamorphosing tadpoles. The most noticeable internal changes during metamorphosis are the shortening and differentiation of the intestine and rectum. During these changes the number and distribution of the *Opalinae* were affected as indicated in Table 1 and Figure 1. Here are presented data from a study of 15 specimens arranged in 3 groups on the basis of length of intestine and presence and length of fore and hind legs. The length of the rectum is difficult to determine because it is tightly coiled, hence the measurements given for this section are only approximate.

1. The five specimens in an "early" stage of metamorphosis were without fore legs and possessed intestines ranging from 320 to 265 mm. in length. In the rectum of these *Opalinae* were present in large

numbers—designated by “many” or “abundant.” No Opalinae were found in the intestines except in one case in which there were many.

2. A second group of five tadpoles are termed “intermediates.” The intestines of these were 245 to 138 mm. in length and there were no forelegs. Opalinae were numerous in the rectum of two, rare in two and absent in one; they were present in very small numbers in the intestines of all five tadpoles.

3. The third group of five were in a “late” stage of metamorphosis. Their intestines ranged from 145 to 38 mm. in length and in all of them the forelegs had emerged. No opalinas were found in the rectum of any of them and in the intestine of only one. This specimen had a comparatively long intestine and only a few ciliates were present.

TABLE 1.—MEASUREMENTS OF METAMORPHOSING TADPOLES OF THE GREEN FROG IN EARLY, INTERMEDIATE AND LATE STAGES SHOWING THE RELATION BETWEEN LENGTH OF INTESTINE AND RECTUM AND NUMBER OF OPALINAE PRESENT

Stage of Meta- morphosis	Length in Mm. of				Length in Mm. of		Number of Opalinae in	
	Body	Tail	Fore Legs	Hind Legs	Intestine	Rectum	Intestine	Rectum
Early.....	24	48	0	20	320	45	Many	Many
Early.....	29	63	0	28	298	35	0	Many
Early.....	28	66	0	30	272	40	0	Abundant
Early.....	25	45	0	12	270	25	0	Abundant
Early.....	27	45	0	22	265	45	0	Many
Intermediate...	29	60	0	36	245	30	Few	Many
Intermediate...	27	53	0	24	220	25	Few	0
Intermediate...	27	61	0	27	215	35	Rare	Rare
Intermediate...	28	55	0	39	170	25	Few	Rare
Intermediate...	28	64	0	33	138	25	Rare	Many
Late.....	28	55	13	40	145	22	Few	0
Late.....	30	64	15	37	130	20	0	0
Late.....	28	59	17	41	70	10	0	0
Late.....	30	57	18	50	44	7	0	0
Late.....	27	50	20	47	38	7	0	0

The other intestinal protozoa differed markedly from Opalina in numbers and distribution. *Nyctotherus* was present in the rectum of all of the 15 tadpoles examined, being numerous in all of the early stages, in two of the intermediates and three of the late stages. No specimens of this form were found in the intestines of the early stages, a few were present in one of the intermediates and several or a few in every one of the late stages. *Trichomonas* occurred in the rectum of all of the early and intermediate stages but in only one of the late stages. It was not numerous in any of them. With the exception of one intermediate, this form was absent from the intestines of all of the 15 examined. *Hexamitus* was found to be abundant or moderate in numbers in the rectum of all of the tadpoles but only a few were present in the intestines of 6 of them.

Data from a larger number of tadpoles would make conclusions more definite, but the findings indicate that Opalinae are normally

present in considerable numbers in the rectum of tadpoles of the green frog from a few days after hatching until the time of metamorphosis. During the period when the intestine decreases in length from about 300 to 150 mm. the Opalinae decrease in numbers or disappear entirely from the rectum and some of them appear to migrate into the intestine. After the forelegs emerge, Opalinae are entirely absent from the rectum and from the intestine of most of the tadpoles. No significant differences, on the other hand, were noted in the numbers and distribution of *Nyctotherus*, *Trichomonas* and *Hexamitus* during metamorphosis.

The number and distribution of Opalinae during the metamorphosis of tadpoles of *Rana pipiens* and of the toad were also obtained in a few cases. These data are of interest for comparison with those noted above because, although adults of both of these species harbor Opalinae they might lose their infection during metamorphosis, as in the green frog, and become reinfected after they are fully transformed. Three metamorphosing tadpoles of *R. pipiens* were examined on July 5 and 3 fully transformed specimens on July 25, 1922. Opalinae were present in the rectum of 5 of the 6 specimens, being absent in 1 of the young frogs; they were also found in the intestine of 4 of the specimens, being absent from 1 of the metamorphosing tadpoles and from 1 of the fully transformed frogs. It seems probable, therefore, that in this species Opalinae persist throughout the period of metamorphosis and that the infection of the adult is a continuation of that of the tadpole. Data obtained from the examination of toad tadpoles indicate that this is true also in this species. Ten toad tadpoles collected on June 12, 1922, were examined on June 28, at which time their average measurements were as follows: body, 8 mm.; tail, 12 mm.; hind legs, 3 mm.; intestine, 47 mm.; rectum, 9 mm. Five of these 10 specimens contained Opalinae. The absence of Opalinae in the other 5 was probably due to loss due to confinement without food for 16 days (see page 55 on effects of starvation on the protozoan fauna of the intestine).

The results of the observations on metamorphosing green frog tadpoles, as regards Opalinae, are shown graphically in Figure 1. In the early stage of metamorphosis the Opalinae are numerous in the rectum but seldom present in the intestine. In the intermediate stages the number in the intestine and rectum is about the same and the total number in the 2 sections probably equals that in the early stages. What apparently has happened is that ciliates have migrated from the rectum into the intestine. In the late stages the Opalinae disappear from the rectum first and then from the intestine.

II. *The Effects of Starvation of the Host on Intestinal Protozoa.*—The factors of the environment of intestinal protozoa differ considerably from those of free-living species. In such an animal as the tadpole

the light that penetrates the intestinal wall must be very feeble; the medium within the intestine is comparatively viscous; the possibility of being swept away as the food masses pass through must be guarded against; the chemical conditions are variable and complex due to digestive juices, the products of digestion, and other biochemical products produced by the host; and locomotion is difficult and requires more energy than in free-living species. Among the important environmental factors is, of course, the food supply. One cannot tell as yet the exact nature of the food of any of the protozoa dealt with in this paper, but there certainly must be a marked change in the intestinal contents of the tadpole under starvation conditions which might affect favorably or unfavorably the protozoan fauna. Tadpoles of the toad and of *Rana*

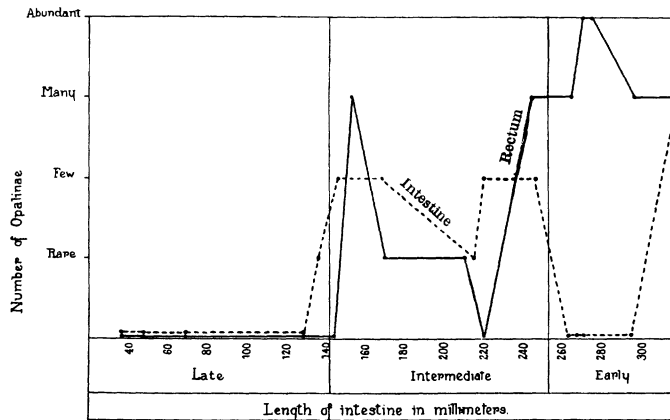


Fig. 1.—Curves showing the comparative numbers of *Opalinae* present in the intestine (broken line) and rectum (solid line) of 15 tadpoles of the green frog in early, intermediate and late stages of metamorphosis.

pipiens and *R. clamitans* were kept for various periods in the laboratory in glass dishes without food, the water being changed frequently thus preventing the continued reingestion of materials that had already passed through the digestive tract. *Opalina*, *Nyctotherus*, *Trichomonas*, and *Hexamitus* were present in almost all tadpoles at the time they were collected from the ponds. Examinations of the rectum and intestine gave, in brief, the following results:

1. *Opalinae* were found in *R. pipiens* up to the 9th day in 3 specimens but was absent in four specimens examined on the 10th, 12th, 22d and 25th days, respectively. They were present in small numbers in 4 specimens of *R. clamitans* examined on the 12th day, although abundant in specimens direct from the ponds. Fourteen toad tadpoles dissected from the 16th to the 37th day revealed *Opalinae* in small numbers in only 2 specimens on the 16th day. Thus lack of food under laboratory conditions appears to be decidedly detrimental to *Opalina*.

2. *Nyctotherus* seems to disappear more rapidly than *Opalina*. None were found in 7 specimens of *R. pipiens* after from 7 to 25 days, nor in 4 specimens of *R. clamitans* after 12 days, and in only 2 of 14 specimens of toad tadpoles after 16 days.

3. *Trichomonas* was not very abundant in any of the tadpoles examined and data regarding their persistence or disappearance are not definite.

4. *Hexamitus* did not seem to suffer particularly in the toad tadpoles, being present in large numbers after 29 and 37 days, respectively, although there was a falling off in numbers in tadpoles of *R. pipiens* in 22 and 25 days and in *R. clamitans* in 12 days. It may be concluded from these data that starvation under laboratory conditions is more or less disadvantageous to the species of protozoa studied. Previous observations on the intestinal fauna of tadpoles has given me the general impression that these protozoa do not disappear at all or at least not so rapidly under laboratory conditions when their hosts are well fed, but I have no definite data to prove this.

III. *The effects of Changes in the Diet of the Host on the Incidence, Distribution and Numbers of Intestinal Protozoa.*—In selecting food substances that may have an influence on the intestinal protozoa of tadpoles, materials from the ductless glands are immediately suggested, because of the remarkable modifications they have been shown to bring about.

Since Gudernatsch (1912) first reported the effects of a diet containing thyroid substance on the metamorphosis of frog tadpoles, many investigators have subjected protozoa to this and to materials from other ductless glands as well as to substances in which vitamins of different types were either present or absent. Many of the efforts in this direction were negative or indefinite but others have been positive. Shumway (1917), for example, has shown conclusively that the division rate of *Paramecium* is increased about 65 per cent. by a diet of desiccated thyroid. The hormone in thyroid has been found to act as a chemical stimulus to other tissues causing increased metabolism. In the case of *Paramecium* this increased metabolism probably results in more rapid growth and hence a higher rate of division.

So far as I know none of the thousands of tadpoles used for experiments on the feeding of glandular substances or on the effects of removing or grafting ductless glands have been examined to see whether any changes had occurred in the intestinal fauna. The questions involved in the investigations described below are: 1. Do glandular and other substances eaten by the host affect the rate of division of intestinal protozoa as they do of free living species? 2. Is the incidence of infection and distribution within the digestive tract changed by

radical changes in the diet of the host? 3. Can the incidence, numbers and distribution of the protozoa be correlated with changes in the host due to modifications of the diet?

The tadpoles of the green frog, *Rana clamitans*, were used as experimental animals and the protozoa studied were principally *Opalina*, *Nyctotherus*, *Trichomonas*, and *Hexamitus*. The green frog, as mentioned above, is of particular interest since the large ciliate, *Opalina*, occurs in large numbers in the tadpoles, but is almost never found in the adults. The principal material used as food was desiccated thyroid substance. This was selected because it has been shown to have a marked influence on the division rate of certain free-living protozoa and on the metamorphosis of tadpoles. The other glandular substances used were thymus, ovary, orchic, prostate, pituitary, and suprarenal.

1. *Preliminary Experiments.*—Several preliminary experiments were carried out in January and March, 1921, for the purpose of perfecting methods and of determining how many tadpoles were necessary to give significant results. One group of 6 tadpoles furnished material for an experiment lasting 9 days (January 19 to 28), and another group of 15 for an experiment lasting 7 days (March 7 to 14). It was found that tadpoles of the green frog when fed on desiccated thyroids mixed with flour began to die on the 7th day and that considerable mortality occurred on the 8th and 9th days. Evidences of external metamorphosis were conspicuous; the body changed considerably in shape and decreased in length about 20 per cent., and hind legs were developed. The most interesting internal change noted was a decrease in the length of the intestine of about 50 per cent. No significant data were obtained regarding the intestinal protozoa.

2. *Further Experiments in 1921 with Desiccated and Powdered Thyroid Glands, Thymus Glands, Ovaries, and Orchic Substance.*—In these experiments the controls were fed on flour that had been made into a paste with water, spread on glass plates to dry, and then crumbled (Swingle, 1918). The experimental animals were fed on 7 parts flour plus 3 parts desiccated and powdered glandular substance prepared in the same way. The tadpoles were kept in circular glass dishes 22 cm. in diameter. The water and food were changed daily, 1 liter of tap water being provided for each dish and an excess of food added. The data obtained are given in Table 2. No very definite method was devised of determining the exact number of protozoa present. The entire rectum of each tadpole was stirred up on a slide with about 0.1 c.c. of normal saline solution and part of this covered with an 18 mm. square cover glass. A binocular dissecting microscope was used to detect the presence of ciliates and a compound microscope with

number 5 ocular and 16 mm. and 4 mm. objectives was then employed to determine the numbers of both ciliates and flagellates.

The tadpoles proved to be favorable material for experimental purposes since very few of them died and the incidence of infection was high; 80 per cent. were infected with *Nyctotherus*, 90 per cent. with *Opalina*, 85 per cent. with *Trichomonas*, and 95 per cent. with *Hexamitus*. A comparison of the controls and experimental tadpoles is possible with the data contained in Table 2.

(a) *Thyroid Experiments*.—Tadpoles fed on desiccated thyroids and examined at the end of 5 days changed both externally and internally as in the preliminary experiments. The rectum contained less material than that of the controls and both the incidence of infection

TABLE 2.—INCIDENCE OF INFECTION AND COMPARATIVE NUMBERS PRESENT OF TWO CILIATES, NYCTOTHERUS AND OPALINA, AND TWO FLAGELLATES, TRICHOMONAS AND HEXAMITUS, IN THE RECTUM OF TADPOLES OF THE GREEN FROG, *Rana clamitans*, AND THE BULL FROG, *R. catesbiana*, AFTER BEING FED ON VARIOUS GLANDULAR SUBSTANCES

Gland- ular Sub- stance	No. of Tad- poles	Nyctotherus			Opalina			Trichomonas			Hexamitus		
		Per Cent. In- fected	Per Cent. with Many	Per Cent. with Few	Per Cent. In- fected	Per Cent. with Many	Per Cent. with Few	Per Cent. In- fected	Per Cent. with Many	Per Cent. with Few	Per Cent. In- fected	Per Cent. with Many	Per Cent. with Few
Control..	20	80	15	65	90	65	25	85	15	70	95	90	5
Thyroid..	27	26	3.7	22	4	0	4	11	0	11	89	52	37
Thymus..	17	76	41.2	35	35	11.7	24	59	12	47	82	59	24
Ovary....	20	75	25	50	45	15	30	85	35	50	85	55	30
Orchie....	17	71	46.9	24	29	5.8	24	71	29	41	29	6	24

and number present decreased for all four protozoa recorded. The incidence of infection with *Nyctotherus* decreased 66 per cent. and the numbers when present decreased about 50 per cent. This result is very different from that obtained when a similar free-living ciliate, *Paramecium*, is fed on thyroid substance, division rate in the latter being accelerated about 65 per cent. (Shumway, 1917). There seems to be no reason why *Nyctotherus* should not react to thyroid feeding by dividing more rapidly the way *Paramecium* does. The difference noted may be due (1) to a change in the thyroid substance before it reaches the rectum such as the absorption of the effective hormone, or (2) to the change in the intestinal tract characteristic of thyroid-fed tadpoles. *Opalina* and *Trichomonas* were almost eliminated from the thyroid-fed tadpoles and *Hexamitus*, although present in almost as many specimens, was not so numerous.

(b) *Thymus Experiments*.—Seventeen tadpoles were examined after six days of feeding on dessicated thymus. *Nyctotherus* decreased slightly in incidence but increased considerably in numbers. *Opalina* decreased about 60 per cent. in incidence and also in numbers. The incidence of *Trichomonas* fell off about 30 per cent., but an increase

in numbers took place. Hexamitus decreased slightly in incidence and considerably in numbers.

(c) *Ovary Experiments*.—In the 20 tadpoles fed on desiccated ovarian substance for six days, *Nyctotherus* decreased slightly in incidence but increased in numbers; *Opalina* decreased 50 per cent. in incidence and also in numbers; *Trichomonas* increased in numbers, and *Hexamitus* decreased slightly in both incidence and numbers.

(d) *Orchic Experiments*.—The 17 tadpoles fed on desiccated orchic substance showed a slight decrease in the incidence of *Nyctotherus* but an increase in numbers; a 66 per cent. decrease in the incidence of *Opalina* and a corresponding decrease in numbers; a slight decrease in the incidence of *Trichomonas* accompanied by an increase in numbers; and about a 70 per cent. decrease in incidence of *Hexamitus* with a great decrease in numbers.

If one considers the four organisms under consideration separately one finds *Nyctotherus* adversely affected by thyroid feeding, and slightly reduced in incidence but increased in numbers, when present, by thymus, ovary, and orchic substance. The incidence of *Opalina* and numbers present decreased considerably in all the experimental tadpoles. Thyroid and thymus feeding reduced radically the incidence of *Trichomonas* but thymus, ovary and orchic substance caused an increase in numbers when present. The incidence of *Hexamitus* was not greatly influenced except by orchic substance which caused a great decrease. The number of *Hexamitus* present decreased in all of the experiments.

The organisms selected for study comprise two species that are holozoic, *Nyctotherus* and *Trichomonas*, and two, whose method of nutrition is osmotic, *Opalina* and *Hexamitus*. The data obtained show that the former suffered less than the latter during the course of the experiments. In tadpoles fed on thymus, ovary, and orchic substance the incidence of infection with *Nyctotherus* was almost as high as in the controls and there was an actual increase in the numbers present. The data show similar results with *Trichomonas* although in thymus-fed tadpoles the incidence fell about 33 per cent. On the other hand, the incidence of *Opalina* in tadpoles fed on these 3 substances fell from 50 to 67 per cent. and a decrease in numbers was observed; and in *Hexamitus*, although the incidence decreased markedly only in orchic-fed animals, the numbers likewise decreased considerably.

The number of tadpoles studied was small and small variation in incidence and number are therefore not significant, but it seems clear that the 2 protozoa that ingest solid particles, *Nyctotherus* and *Trichomonas*, were, on the whole, not unfavorably affected by the diets used and may even have been stimulated to more rapid growth and division. The other 2 protozoa with the osmotic type of nutrition, *Opalina*

and Hexamitus, seem to have been acted upon unfavorably, due probably to the absorption of deleterious substances in solution.

3. *Experiments in 1922 with Thyroid Glands, Prostate Glands, Pituitary Glands, Suprarenals, Orchic Substance, and Meat.*—Sixty one-year-old tadpoles of the green frog and bull frog were used for each diet, 20 being placed in each dish and fed as described above. The results of these extensive feeding experiments are not as satisfactory as expected because the incidence of infection with *Nyctotherus* and *Opalina* was very low, namely, 15 per cent. with the former and 5 per cent. with the latter. Data regarding these two species have been omitted from Table 3 on this account since the results were not significant. For obtaining a basis for the comparison of the numbers of protozoa present, the number of each species was counted in 10 fields of the rectal contents of each tadpole using a 4 mm. objective and a No. 10 ocular and an average taken. The data show that *Trichomonas*

TABLE 3.—INCIDENCE OF INFECTION AND COMPARATIVE NUMBERS PRESENT OF TWO FLAGELLATES, *TRICHOMONAS* AND *HEXAMITUS*, IN THE RECTUM OF TADPOLES OF THE GREEN FROG AND BULL FROG, AFTER BEING FED ON VARIOUS SUBSTANCES

Substance	Number of Tadpoles	Trichomonas		Hexamitus	
		Per Cent. Infected	Average Number per Field	Per Cent. Infected	Average Number per Field
Control.....	39	92	6	90	9.3
Thyroid.....	50	16	0.27	76	3.2
Prostate.....	19	50	0.2	85	9.0
Pituitary.....	20	90	5	100	35.2
Suprarenal.....	10	80	2.34	90	15.5
Orchic.....	10	100	6.5	100	34.2
Meat.....	25	75	4.1	100	16.7

suffered severely in the thyroid-fed tadpoles, both in incidence of infection and in numbers present. Decreases almost as great occurred in prostate-fed tadpoles. The intestine of tadpoles fed on these substances are profoundly affected, undergoing rapid metamorphosis (Swingle, 1918; Hegner, 1922) a phenomenon that may account for these decreases rather than the direct action of the ingested substances. In no case does the experimental diet seem to have had a particularly favorable effect on *Trichomonas*. *Hexamitus* also decreased in the thyroid-fed tadpoles but not to such an extent as did *Trichomonas*. The other diets seem to have favored its growth and multiplication, especially pituitary and orchic substance. This result does not agree with the experiments of 1921 (Table 2) in which a marked decrease in incidence and numbers of *Hexamitus* was noted in tadpoles fed on orchic substance.

4. *Further Experiments in 1922 with Thyroid Substance.*—During the month of June, 1922, opportunity was afforded to carry on another series of experiments with thyroid substance at Cornell University,

Ithaca, N. Y. One-year-old green frog tadpoles were collected on June 14 and fed on thyroid substance daily until June 18 or 19. Controls collected on the same date were examined on June 17. During the 4 or 5 days of the experiment evidences of both external and internal metamorphosis appeared. Table 4 gives measurements of 10 specimens each of normal and thyroid-fed tadpoles. The most noticeable changes are an average decrease of about 20 per cent. in the length of the body and of about 40 per cent. in the length of the tail of the thyroid-fed specimens and an average increase in the length of the hind legs of these same specimens of about 93 per cent.

The only internal changes studied were those involving the digestive tract. The rectum was difficult to measure on account of its coils and hence the data with regard to this section of the alimentary canal are

TABLE 4.—MEASUREMENTS IN MM. OF TEN SPECIMENS EACH OF CONTROL TADPOLES OF THE GREEN FROG AND TADPOLES THAT HAD BEEN FED ON THYROID SUBSTANCE FOR FIVE DAYS

	Nyctotherus Length of Body		Opalina Length of Tail		Trichomonas Length of Hind Legs		Hexamitus Total Length	
	Range	Average	Range	Average	Range	Average	Range	Average
Controls	20-26	21.4	27-46	32.8	2-3.5	2.75	47-72	54.2
Thyroid-fed.....	15-19	17.2	13-29	19.8	3-8	5.3	29-48	37

TABLE 5.—MEASUREMENTS IN MM. OF THE INTESTINE AND RECTUM OF TWENTY-FIVE SPECIMENS EACH OF CONTROL TADPOLES OF THE GREEN FROG AND TADPOLES THAT HAD BEEN FED ON THYROID SUBSTANCE FOR FOUR OR FIVE DAYS

	Length of Intestine		Length of Rectum	
	Range	Average	Range	Average
Normal.....	180-309	214.6	18-40	25.2
Thyroid-fed.....	82-188	112.2	10-25	14

only approximate. The differences in the measurements of the intestine and rectum of twenty-five specimens each of control and thyroid-fed tadpoles are shown in Table 5. The intestine and rectum of the thyroid-fed tadpoles decreased about 50 per cent. in length during the 4 or 5 days they were fed on this diet.

Number and distribution of *Nyctotherus*, *Opalina*, *Trichomonas*, and *Hexamitus*. As in previous experiments the entire contents of the rectum of each specimen was thoroughly mixed with a measured amount of normal saline solution; in these experiments 0.1 c.c. was used. Counts were made as follows. The diluted rectal contents were spread over approximately one-half of a 1 by three inch slide; an 18 mm. square cover slip was then placed on part of it; the average number of *Opalinae* present in ten microscopic fields using a 16 mm. objective and a No. 10 ocular was obtained, and of *Trichomonas* and *Hexamitus*

in ten microscopic fields using a 4 mm. objective and No. 10 ocular. The results are shown in Table 6 and in Figures 2 and 3.

According to the data in Table 6 the feeding of thyroid substance to green frog tadpoles has a decidedly unfavorable effect on the protozoan inhabitants of the rectum. *Nyctotherus* and *Trichomonas* were almost eliminated; and although the incidence of infection with *Opalina* and *Hexamitus* decreased only slightly, there was a very striking decrease in the numbers of these species when present. These results correspond fairly well with those obtained in the previous experiments described above. No better tadpoles for experimental purposes could be obtained than those used in this series since there was 100 per cent. of infection with all of the protozoa studied. Figure 2 shows by means of curves the relations between the length of the

TABLE 6.—COMPARATIVE NUMBERS OF NYCTOTHERUS, OPALINA, TRICHOMONAS, AND HEXAMITUS IN THE RECTUM OF TWENTY-FIVE SPECIMENS EACH OF CONTROL TADPOLES OF THE GREEN FROG AND OF TADPOLES THAT HAD BEEN FED ON THYROID SUBSTANCE FOR FOUR OR FIVE DAYS
(For method of obtaining these numbers see text)

	Per Cent. In- fected	Number per Field		Per Cent. In- fected	Number per Field		Per Cent. In- fected	Number per Field		Per Cent. In- fected	Number per Field	
		Range	Aver- age		Range	Aver- age		Range	Aver- age		Range	Aver- age
Normal.....	100	Pres- ent (1)	Pres- ent (1)	100	1-30	7.16	100	3-16	7.28	100	2-20	8.48
Thyroid-fed...	8	Pres- ent (1)	Pres- ent (1)	84	0.1-3	0.44	20	0-8 (2)	Few (2)	92	0-6 (3)	Few (3)

1. *Nyctotherus* was present in every specimen but too few in numbers to give a satisfactory count.

2. *Trichomonas* was present in moderate numbers in only three specimens, and rare in two other specimens.

3. *Hexamitus* was present in moderate numbers in 12 specimens but rare or few in number in 11 other specimens.

rectum and number of *Opalinae* present in the rectum of the twenty-five control and twenty-five thyroid-fed tadpoles, and brings out clearly the fact that a diet of thyroid causes a marked decrease in the length of the rectum and a corresponding decrease in the number of *Opalinae* present.

Protozoa in the Intestine.—The intestine of the 25 control and 25 thyroid-fed tadpoles were also carefully examined with results that are of considerable interest. It was found that in normal tadpoles the 4 protozoa studied were almost entirely limited to the rectum. One or 2 *Opalinae* were observed in the intestinal contents of 7 of the 25 control specimens; these were probably transients and not regular inhabitants of this part of the digestive tract. *Hexamitus* in considerable numbers was encountered in 1 tadpole and *Giardia agilis* occurred in many of the tadpoles. In the intestine of the thyroid-fed tadpoles, on

the other hand, there was an incidence of infection of 100 per cent. with *Opalina*, and an average of 1.94 per field; 100 per cent. of infection with *Hexamitus*, many being present in all but 3 specimens; and 25 per cent. of infection with *Trichomonas*, although very few in numbers. Examinations of various parts of the intestine revealed a rather constant number of the various species throughout. A comparison of the effects of thyroid feeding and of natural metamorphosis reveals the fact that in both cases the *Opalinae* are similarly distributed. These data indicate that changes in the intestine due to a thyroid diet rather than the direct action of the thyroid substance has caused an emigration of the

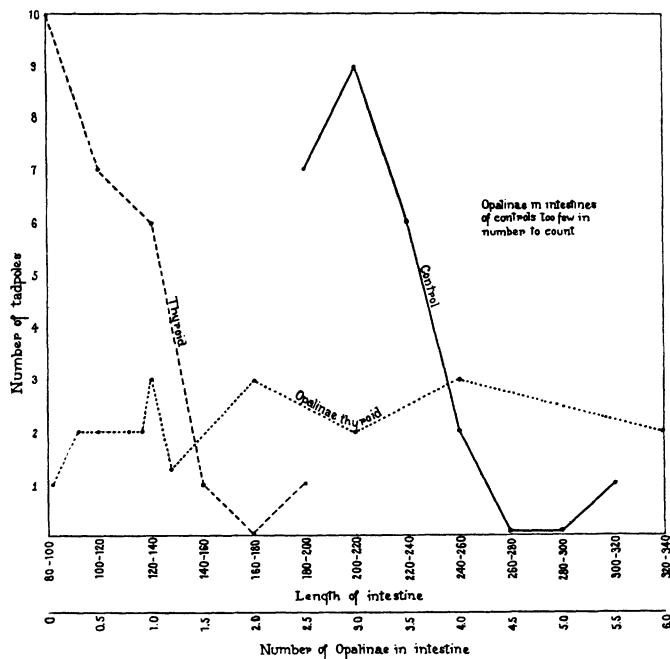


Fig. 2.—Curves showing the length of the rectum and comparative numbers of *Opalinae* present in 25 control and 25 thyroid-fed tadpoles of the green frog.

protozoa from the rectum and their distribution throughout the intestine, which is not their normal habitat.

5. *Experiments with an Animal Diet.*—Metcalf (1920, in litt.) has suggested that the disappearance of *Opalinae* in the green frog might take place "at the time of change from vegetable to animal diet." Tadpoles are essentially vegetable feeders but will take animal food when available. They will devour dead animals that they may find in their habitat and will feed on animal food entirely in the laboratory. It has been shown above that the *Opalinae* disappear from the tadpoles during metamorphosis but before transformation is completed. At this

time the diet appears to be the same as that of the younger tadpoles, the change to the purely animal diet not occurring until the young frog leaves the water. The infection thus disappears before the food becomes animal in nature. My experiments designed to test the effects of an animal diet have not been conclusive. Tadpoles of the green frog, when fed on dessicated glandular substances, have been shown above to lose their infections with Opalinae entirely or to be infected with lesser numbers. Beef meal was tried as a food in one experiment but most of the tadpoles died within 3 days. The 3 that survived were all infected with Opalinae, but the numbers of these were less than in control tadpoles. Twenty-five tadpoles of the green frog were fed on a diet consisting of the following ingredients: liver, 35 per cent.; beef, 27; dry ox blood, 18; lard, 20; and calcium carbonate, 1.5. A similar diet has been found by McCollum and Simmons to be favorable for the growth and reproduction of rats. These tadpoles, as shown in Table 3, did not exhibit any great decrease in the incidence of infection nor the numbers present of *Trichomonas* and *Hexamitus*. Unfortunately the controls were very lightly infected with *Opalina* and *Nyctotherus* and hence no conclusions could be reached regarding these forms.

A suggestion has been made to me by Metcalf (1920, in litt.) to account for the presence of Opalinae in the tadpoles and their absence from the adults of the green frog, namely, that "the tadpoles of *R. clamitans* may become infected with cysts of a species of Opalinid not found in the adult *R. clamitans* and that they thrive for a time and later in the life of the host disappear." This suggestion may be tested by experiment. Metcalf, indeed, "was able to infect several species of *Anura* with cysts of unaccustomed Opalinids and the Opalinids thrived in the tadpoles of the unaccustomed hosts for a period of about 4 months." The tadpoles had to be killed at the end of this period. Since adults of the green frog do not usually harbor any species of *Opalina*, any species that occurs in the tadpole of this species of frog would be one "not found in the adult." The difference between the green frog and certain other species of frogs and toads is that no species of Opalinae have become regular inhabitants of the intestine of adults of the former, whereas, each of the latter is infected by one or more species, but refractory toward other species. The problem is the fundamental one of specificity in parasitism.

SUMMARY

1. The study of the incidence, distribution and numbers of the ciliate *Opalina*, in the intestine and rectum of the tadpoles of *Rana clamitans* indicates (a) that these tadpoles are very susceptible to infection with

Opalinae, the ciliates being numerous in specimens from 10 days old to the time of metamorphosis; (b) that during early stages of metamorphosis Opalinae are numerous in the rectum but not in the intestine; (c) that during intermediate stages part of the Opalinae migrate into the intestine, and (d) that in late stages all of the Opalinae disappear from the rectum and later from the intestine. Thus no young green frogs are infected with this protozoon and no species of Opalina seems to have succeeded in maintaining itself in the intestine or rectum of adult frogs of this species.

2. The ciliate, *Nyctotherus*, and flagellates, *Trichomonas* and *Hexamitus*, were found in all stages in the metamorphosis of green frog tadpoles and infection with these protozoa is probably continuous from tadpole to adult.

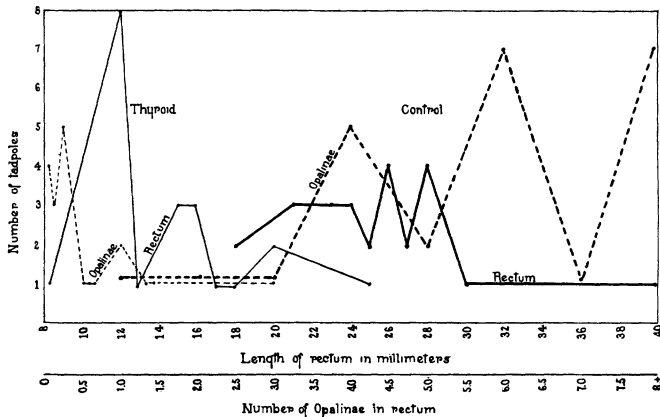


Fig. 3.—Curves showing the length of the intestine of 25 control and 25 thyroid-fed tadpoles of the green frog, and the number of Opalinae in the intestine of the latter.

3. Both adults and tadpoles of *Rana pipiens* and the toad are infected with Opalinae. This infection is continuous from tadpole to adult, and not an infection of the tadpole which disappears during metamorphosis and is followed by a reinfection of the adult.

4. Lack of food is disadvantageous to the intestinal protozoa of tadpoles of the green frog, leopard frog, and toad. Opalina was rarely present after a period of two weeks; *Nyctotherus* disappeared usually in about 1 week; and *Hexamitus*, although persisting in considerable numbers in toad tadpoles, decreased in both *Rana pipiens* and *R. clamitans*.

5. Preliminary experiments in 1921 on the effects of desiccated thyroid substance on the intestinal protozoa of green frog tadpoles

showed that considerable mortality occurs after one week. During this period external metamorphosis advances rapidly and the length of the intestine decreases about 50 per cent.

6. Further experiments in 1921 with desiccated thyroid, thymus, ovaries and orchic substance show that thyroid feeding results in the decrease both in incidence and numbers of *Opalina*, *Nyctotherus*, *Trichomonas* and *Hexamitus*. The 2 holozoic forms, *Nyctotherus* and *Trichomonas*, undergo a decrease in incidence but an increase in numbers when their tadpole hosts are fed on thymus, ovarian, or orchic substance. The 2 forms with osmotic nutrition, *Opalina* and *Hexamitus*, decreased in both incidence and numbers in tadpoles fed on these 3 substances.

7. Experiments in 1922 with thyroid, prostate, pituitary, suprarenal and orchic substances were only partially successful because of the low incidence of infection with *Opalina* and *Nyctotherus*. *Trichomonas* was adversely affected noticeably by diets of thyroid and prostate substance, both as regards incidence and numbers present and was reduced slightly in tadpoles fed on suprarenal substance. Very little difference was produced by diets containing pituitary and orchic substances. *Hexamitus* was influenced adversely very slightly by prostate and considerably by thyroid substance. An actual increase in numbers of *Hexamitus* was recorded in tadpoles fed on pituitary, suprarenal and orchic substances and an increase in incidence in tadpoles fed on pituitary and orchic substances. It is interesting to note that the 2 substances that are known to bring about marked modifications in the intestine, namely, thyroid and prostate, affect adversely the intestinal protozoa.

8. Further experiments in 1922 with thyroid substance furnished more extensive data. After thyroid feeding for 4 or 5 days *Nyctotherus* was almost eliminated from the rectum; *Opalina* decreased 16 per cent. in incidence and very markedly in numbers; *Trichomonas* decreased 80 per cent. in incidence and only a few were present; and *Hexamitus* decreased 8 per cent. in incidence and very greatly in numbers. The intestines of the control tadpoles were almost entirely free from protozoa, but in the thyroid-fed animals the intestine of every specimen was heavily infected with both *Opalina* and *Hexamitus* and 25 per cent. were infected with *Trichomonas*. Apparently the changes in the alimentary canal due to thyroid feeding brought about a change in distribution of these protozoa similar to that found in tadpoles undergoing metamorphosis. This adds weight to the hypothesis that it is not the direct effect of the food of the host but the changes in the digestive tract due to this food that influence the incidence and numbers of the protozoa present.

9. Tadpoles of the green frog were fed on an animal diet that has been proved to be satisfactory for the growth and reproduction of rats. No marked changes in the incidence and numbers of *Trichomonas* and *Hexamitus* were induced by this diet.

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